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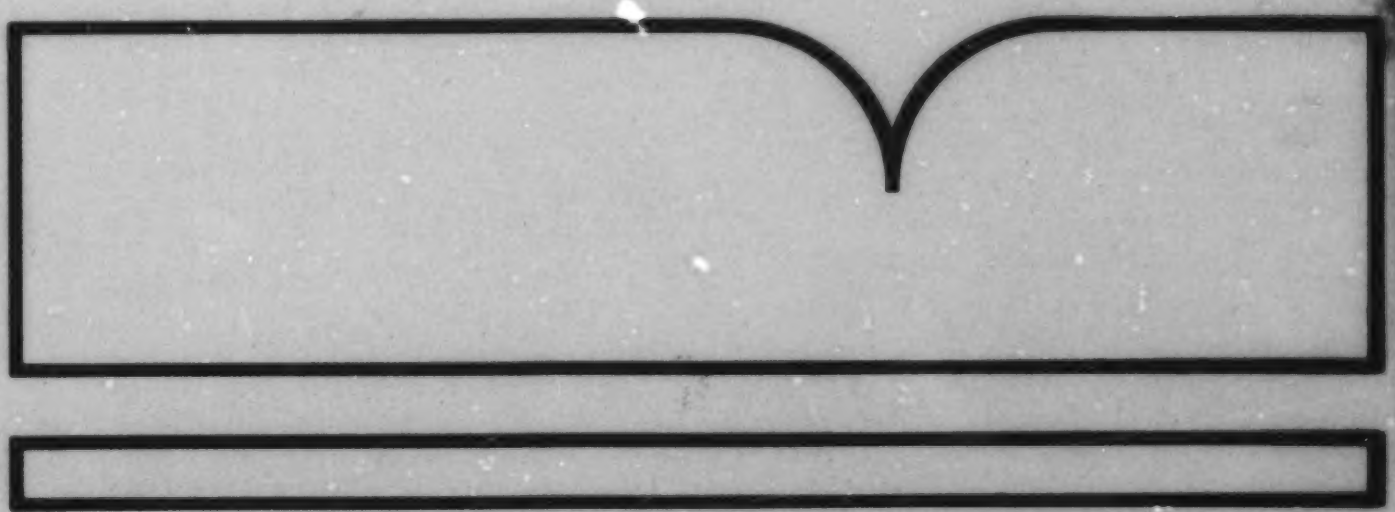

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16. ABSTRACT

Approximately 1000 urine samples were collected from the general adult U.S. population, and analyzed for cadmium, beta$_2$-microglobulin, specific gravity, and creatinine. The distributions of urinary cadmium and beta$_2$-microglobulin levels were quantified for use as benchmarks against which the effects of environmental cadmium exposure may be measured. Urinary cadmium is significantly higher in females than males, and increases significantly with age. Urinary beta$_2$-microglobulin is significantly higher in males than females, is probably related to age, and does not significantly increase with increased urinary cadmium (at the levels found). Since urinary creatinine is lower in females than males and is a function of age, specific gravity is concluded to be the most straightforward and practical parameter to use in urine concentration adjustment.

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URINARY CADMIUM AND BETA$_2$-MICROGLOBULIN: NORMAL VALUES AND CONCENTRATION ADJUSTMENT

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Approximately 1000 urine samples were collected from the general adult U.S. population and were analyzed for cadmium, beta$_2$-microglobulin, specific gravity, and creatinine. The distributions of urinary cadmium and beta$_2$-microglobulin levels were quantified for use as benchmarks against which the effects of environmental cadmium exposure may be measured. Urinary cadmium is significantly higher in females than males, and increases significantly with age. Urinary beta$_2$-microglobulin is significantly higher in males than females, is probably related to age, and does not significantly increase with increased urinary cadmium (at the levels found). Since urinary creatinine is lower in females than males and is a function of age, specific gravity is concluded to be the most straightforward and practical parameter to use in data adjustment for urine concentration.

INTRODUCTION

Increased human exposure to cadmium as an environmental pollutant through food, water, air, and smoking has raised concern about its toxic renal effects. Soil cadmium concentrations are naturally low, but may be augmented by airborne fallout, phosphate fertilizers, and municipal sewage sludges. Many vegetable crops tend to accumulate the element from soils, and food ingestion is the major source of cadmium exposure for the general population, with smoking a close second (Ryan et al., 1982). Thus, the accelerating trend toward the application of municipal sludges on land—often agricultural land—may result in increased human exposure to cadmium through the food supply.

Studies by the U.S. Food and Drug Administration and the U.S. Environmental Protection Agency suggest that the cadmium intake by

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Americans through food and water may already be approaching the maximum intake of 57–71 μg/d recommended by the World Health Organization (Kowal et al., 1979). As cadmium accumulates in the body, a threshold concentration is reached in the renal cortex, upon which proteinuria is initiated; estimates of this threshold vary from 100 to 470 μg/g wet weight (Ellis et al., 1981). The earliest indicator in the urine of cadmium-induced renal tubular injury is the presence of elevated levels of the low-molecular-weight proteins $beta_2$-microglobulin, $alpha_2$-microglobulin or retinol-binding protein, and post-gamma-protein (Bernard et al., 1980). Of these, $beta_2$-microglobulin has been the most widely used and is the best characterized (Poulik et al., 1979). Urinary cadmium is a useful index of long-term cadmium exposure and the consequent accumulated body burden (Roels et al., 1981; Nogawa et al., 1979a).

With the factor of safety so small, it is prudent to explore whether a segment of the population is already showing signs of excess cadmium absorption as evidenced by elevated urinary $beta_2$-microglobulin. In this study almost 1000 urine samples were collected from the general adult population and were analyzed for cadmium, $beta_2$-microglobulin, specific gravity, and creatinine. The objectives of the study were (1) to quantify the distributions of urinary cadmium and $beta_2$-microglobulin levels in the adult American population, for use as benchmarks against which the effects of environmental cadmium exposure may be measured; (2) to explore the relationships between these levels and sex and age; and (3) to evaluate data adjustment for urine concentration using the two most commonly employed parameters, specific gravity and creatinine.

[Preliminary results of this study have been reported at the Third International Cadmium Conference (Kowal and Kraemer, 1982).]

METHODS

Subjects

As part of a collaborative study between the National Center for Health Statistics (NCHS) and the U.S. Environmental Protection Agency (EPA), personnel of the NCHS National Health and Nutritional Examination Survey II (NHANES II) collected samples of randomly voided urine from approximately 1000 sample persons during 1978–1979. Thus these persons were a subsample of the total NHANES II surveyed population. The people were of both sexes, 20–74 years of age, and from selected counties in 9 states (Arkansas, Illinois, Indiana, Michigan, New Jersey, New York, Ohio, Pennsylvania, and Texas). Although the results are not strictly representative of the national population, the sample persons do represent a broad cross-section of the United States population with respect to geography, occupational history, income group, race, and ethnic groups.

The total NHANES II survey was a weighted, multistage, clustered probability sample that represents the general U.S. population. Persons less

than 6 years old, greater than 59 years old, and residing in poverty areas were oversampled in NHANES II, so that the results should be interpreted by age-class and economic status, rather than overall averages. This paper presents the preliminary findings on levels of urinary cadmium and beta$_2$-microglobulin, and future analyses will relate these findings to associated socioeconomic, geographic, medical, and nutritional parameters.

Urine Analysis

Urine samples were frozen with dry ice within 1 h of collection and were shipped frozen to a clinical laboratory (Swedish Hospital Medical Center, Seattle, Wash.), where they were analyzed for cadmium, beta$_2$-microglobulin, specific gravity, and creatinine.

Cadmium analysis was performed by chelation and extraction. A 1-ml portion of 2% sodium *N,N*-diethyldithiocarbamate was mixed with 5 ml urine in a centrifuge tube. Methyl isobutyl ketone (3 ml) was added, and the tube was mixed, centrifuged for 5 min, and separated. The organic phase was then analyzed for cadmium by flameless atomic absorption spectrometry, using a Perkin-Elmer 306 instrument with an HGA-2100 graphite furnace. A calibration curve was made from spiked composite urine. Analysis was done in duplicate, with controls in each run, and accuracy of calibration was assessed using National Bureau of Standards material SRM 1643.

Beta$_2$-microglobulin analysis was performed, in duplicate, by radioimmunoassay (Phadebas β_2-Microtest, Pharmacia Diagnostics).

Specific gravity was measured by refractometer, and creatinine by Jaffe's method on a Technicon SMAC.

Statistical Analysis

All urinary cadmium and beta$_2$-microglobulin sample data were analyzed in three forms: (1) unadjusted for urine concentration, (2) adjusted to a specific gravity of 1.020 [i.e., multiplied by 0.020/(sample specific gravity − 1.000)], and (3) adjusted to a per gram creatinine basis. Natural-logarithm-transformed (after concentration adjustment) data were used in all analyses, since both urinary cadmium and beta$_2$-microglobulin appear to be lognormally distributed (subsequently confirmed by analysis).

To quantify the distribution of urinary cadmium and beta$_2$-microglobulin levels, geometric means and geometric standard deviations[1]

[1] Explanation of geometric mean and geometric standard deviation: If the values, x_j, of a random variable are lognormally distributed, the log-transformed values, ln x_j, are normally distributed. The mean and standard deviation of this latter distribution are μ and σ, respectively. Returning to the dimensions of the original values, $\exp(\mu)$ is the "geometric mean" (GM) and $\exp(\sigma)$ is the "geometric standard deviation" (GSD). To generate confidence limits, the standard normal deviate, z, is used: $\ln x = \mu + z\sigma$. Returning to the dimensions of the original values, $x = \exp(\mu + z\sigma)$ or $x = \mathrm{GM} \cdot \mathrm{GSD}^z$. (It should be kept in mind that z can be negative, for the lower confidence limit, implying division in the latter equation.)

were calculated by sex, age decade, and sex–age decade combinations. These 1000 urine samples have been treated in this paper as a simple random sample. Hence the calculation of sample estimates of urinary cadmium and beta$_2$-microglobulin levels may be different from national estimates, since the determinations were not done in all NHANES II sample persons. Specifically, the weighted variance estimates would be approximately 1.5–4 times the simple random sample variance estimates (based upon NCHS experience).

To explore the relationships between urinary cadmium and beta$_2$-microglobulin levels, and sex and age, polynomial regression and analysis of variance (ANOVA) were performed. A polynomial regression analysis of urinary cadmium against age was performed separately for each sex. A polynomial regression analysis of urinary beta$_2$-microglobulin against urinary cadmium, age, and $(\text{age})^2$ was performed separately for each sex.

Analysis of variance (ANOVA) was performed for urinary cadmium and beta$_2$-microglobulin, with sex and age decade as independent variables, and Duncan's multiple range test was used to make multiple comparisons among age decades if age was statistically significant. The test for homogeneity of variance (Bartlett's test) failed, and thus a nonparametric analysis was required. The distribution-free multiple comparisons based on Kruskal–Wallis rank sums (Hollander and Wolfe, 1973) were used for this purpose.

To evaluate the two methods of data adjustment for urine concentration, several tests were performed. Correlation coefficients were calculated between specific gravity and creatinine. ANOVAs were performed with specific gravity, creatinine, and specific-gravity-adjusted creatinine as the response variables, and sex and age as the classification variables. Again, because of failure of homogeneity of variance, nonparametric analyses had to be performed for the creatinine data. The 5% level of significance was used in the interpretation of all statistical tests.

RESULTS AND DISCUSSION

Urinary Cadmium

The frequency distributions of levels of urinary cadmium, summed over both sexes and all ages, are tabulated in Table 1. By checking the log-transformed variables (unadjusted, specific-gravity-adjusted, and creatinine-adjusted urinary cadmium) and their residuals from ANOVA, it was shown that they are lognormally distributed (Fig. 1). Lognormal distributions have been found to be common for cadmium data (Kowal et al., 1979). It is evident that low levels predominate in the population, but that a small fraction of the population may have quite high levels. Lauwerys et al. (1979) and Roels et al. (1981) have suggested a critical urinary cadmium level of 10 μg/g creatinine, below which the probability of developing

TABLE 1. Frequency (F) and Cumulative Frequency (CF) Distributions (%) of Urinary Cadmium

Cadmium (μg/l or μg/g creatinine)	Unadjusted ($n = 982$)		Specific-gravity-adjusted ($n = 982$)		Creatinine-adjusted ($n = 981$)	
	F	CF	F	CF	F	CF
0- <0.1	6.9	6.9	3.0	3.0	3.8	3.8
0.1- <0.2	8.9	15.8	4.5	7.4	7.5	11.3
0.2- <0.3	11.3	27.1	6.3	13.7	8.4	19.7
0.3- <0.4	8.5	35.5	6.5	20.3	7.2	26.9
0.4- <0.5	8.4	43.9	7.7	28.0	8.9	35.8
0.5- <0.6	6.8	50.7	6.2	34.2	7.0	42.8
0.6- <0.7	7.6	58.4	6.6	40.8	7.6	50.5
0.7- <0.8	4.9	63.2	6.2	47.0	5.3	55.8
0.8- <0.9	5.2	68.4	5.1	52.1	5.1	60.9
0.9- <1.0	3.3	71.7	4.3	56.4	4.8	65.6
1.0- <1.1	3.6	75.3	4.7	61.1	4.3	69.9
1.1- <1.2	3.5	78.7	4.2	65.3	3.4	73.3
1.2- <1.3	2.3	81.1	4.3	69.6	2.9	76.1
1.3- <1.4	1.7	82.8	3.0	72.5	2.3	78.5
1.4- <1.5	1.6	84.4	2.4	74.9	3.0	81.4
1.5- <1.6	2.2	86.7	2.1	77.1	1.4	82.9
1.6- <1.7	1.5	88.2	1.8	78.9	2.1	85.0
1.7- <1.8	1.1	89.3	2.4	81.4	1.4	86.4
1.8- <1.9	1.0	90.3	1.4	82.8	1.0	87.5
1.9- <2.0	1.0	91.3	1.9	84.7	1.4	88.9
2.0- <2.2	1.6	93.0	3.1	87.8	2.2	91.1
2.2- <2.4	1.1	94.1	2.9	90.6	2.1	93.3
2.4- <2.6	1.7	95.8	1.4	92.1	1.2	94.5
2.6- <2.8	0.5	96.3	1.4	93.5	0.8	95.3
2.8- <3.0	0.9	97.3	0.9	94.4	0.5	95.8
3.0- <3.2	0.4	97.7	0.7	95.1	0.5	96.3
3.2- <3.4	0.4	98.1	1.2	96.3	0.2	96.5
3.4- <3.6	0.5	98.6	0.4	96.7	0.4	96.9
3.6- <3.8	0.1	98.7	0.6	97.4	0.2	97.1
3.8- <4.0	0.1	98.8	0	97.4	0.1	97.2
4.0- <5.0	0.6	99.4	0.8	98.2	0.6	97.9
5.0-<10.0	0.2	99.6	1.2	99.4	1.4	99.3
10.0-<20.0	0.2	99.8	0.2	99.6	0.3	99.6
20.0-<30.0	0	99.8	0.1	99.7	0.2	99.8
30.0-<40.0	0.1	99.9	0	99.7	0	99.8
40.0-<50.0	0.1	100.0	0.2	99.9	0.1	99.9
50.0-<60.0	–	–	0	99.9	0	99.9
60.0-<70.0	–	–	0.1	100.0	0	99.9
70.0-<80.0	–	–	–	–	0.1	100.0

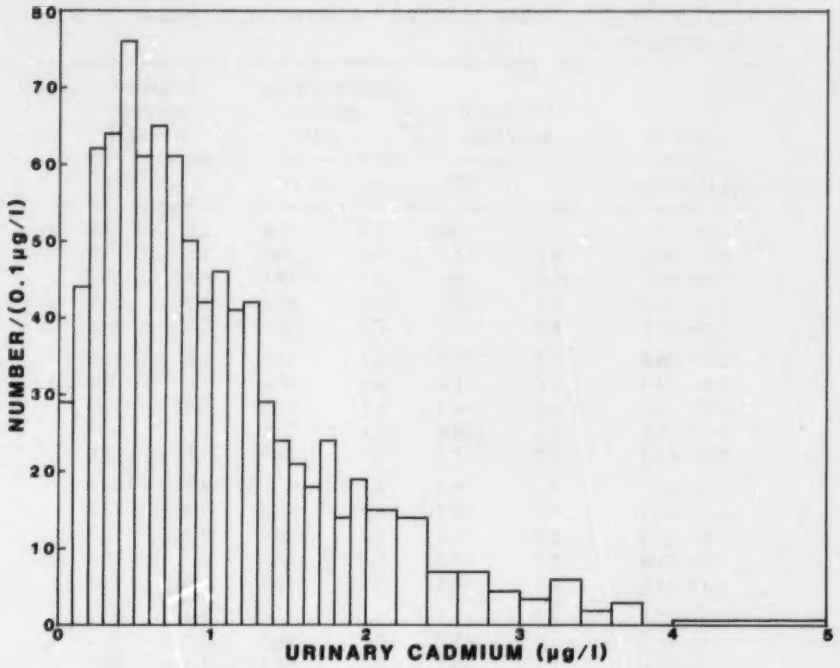

FIGURE 1. Frequency distribution of specific-gravity-adjusted urinary cadmium: $n = 982$, number in range 5–70 µg/l = 18.

cadmium-induced renal dysfunction in male cadmium workers appears to be very low. In our sample, 7 people (0.7%) fall above this critical level. Using $beta_2$-microglobulinuria (i.e., urinary $beta_2$-microglobulin exceeding 5 mg/l) as an index of renal effects, Nogawa et al. (1979b) found 3.2 µg cadmium/g creatinine and 5.2 µg cadmium/g creatinine to correspond with 1% prevalence rates of renal effects in men and women, respectively, living in a cadmium-polluted area of Japan. In our sample, 11 males (2.3%) and 14 females (2.8%) fall above these critical levels of urinary cadmium; none of them has $beta_2$-microglobulinuria as defined by Nogawa et al.

The calculated geometric means and geometric standard deviations of urinary cadmium appear in Table 2, grouped by sex, age decade, and sex-age decade combinations. Our urinary cadmium (specific-gravity-adjusted) estimates of 0.73 (male) and 0.86 (female) µg/l are not too different from our earlier finding of 0.48–0.61 µg/l in a different and smaller U.S. population sample (Kowal et al., 1979). These values are also similar to those found in Sweden (Elinder et al., 1978); Japanese values appear to be higher (Tsuchiya, 1978). Bernard et al. (1979) have found a level of 0.82 µg/g creatinine in unexposed male Belgian workers, somewhat higher than ours.

Comparison of the specific-gravity-adjusted with the unadjusted levels suggests that females have more dilute urine than males, since the male/female ranking reverses upon specific-gravity-adjustment. Likewise, comparison of the creatinine-adjusted with the specific-gravity-adjusted levels suggests that females have lower urine creatinine than males. All the age results suggest a rise in urinary cadmium levels to the sixties, and a fall in concentration thereafter (Fig. 2).

The polynomial regression analysis of urinary cadmium against age resulted in the coefficients and significance levels presented in Table 3. Cubic equations did not increase the explained variation (r^2), and greatly raised p values for most coefficients; thus, quadratic equations appear to be most appropriate to describe the relationship with age. The results indicate that the rise in urinary cadmium with age is statistically significant, and that the fall in the seventies is significant [negative $(age)^2$ coefficient] for females but not for males. Adjusting for urine concentration with specific gravity approximately doubles the explained variation (r^2) over the analysis with unadjusted data. Adjusting with creatinine

TABLE 2. Geometric Means (and Geometric Standard Deviations) of Urinary Cadmium

	Unadjusted		Specific-gravity-adjusted		Creatinine-adjusted	
Parameter	μg/l (GSD)	n	μg/l (GSD)	n	μg/g creatinine (GSD)	n
Sex						
Male	0.552 (2.9)	484	0.728 (2.6)	484	0.554 (2.7)	484
Female	0.490 (3.0)	498	0.863 (2.7)	498	0.779 (2.7)	497
Age						
20–29	0.316 (3.0)	222	0.432 (2.7)	222	0.319 (2.7)	222
30–39	0.456 (3.2)	141	0.704 (2.8)	141	0.538 (2.7)	140
40–49	0.496 (3.0)	142	0.815 (2.6)	142	0.700 (2.7)	142
50–59	0.608 (2.9)	117	0.986 (2.4)	117	0.896 (2.3)	117
60–69	0.761 (2.6)	272	1.163 (2.3)	272	1.032 (2.3)	272
70–74	0.609 (2.2)	88	0.977 (1.9)	88	0.843 (1.8)	88
Age (males)						
20–29	0.296 (2.8)	120	0.366 (2.5)	120	0.261 (2.5)	120
30–39	0.497 (3.0)	63	0.648 (2.5)	63	0.441 (2.4)	63
40–49	0.612 (2.8)	69	0.829 (2.5)	69	0.646 (2.6)	69
50–59	0.688 (2.9)	55	0.949 (2.4)	55	0.770 (2.6)	55
60–69	0.791 (2.7)	131	1.058 (2.4)	131	0.863 (2.4)	131
70–74	0.763 (2.1)	46	1.057 (1.9)	46	0.822 (1.6)	46
Age (females)						
20–29	0.340 (3.3)	102	0.526 (2.8)	102	0.405 (2.9)	102
30–39	0.425 (3.4)	78	0.753 (3.0)	78	0.635 (2.9)	77
40–49	0.407 (3.1)	73	0.802 (2.8)	73	0.756 (2.8)	73
50–59	0.545 (2.9)	62	1.021 (2.4)	62	1.025 (2.1)	62
60–69	0.733 (2.5)	141	1.271 (2.2)	141	1.220 (2.2)	141
70–74	0.475 (2.2)	42	0.897 (1.8)	42	0.868 (1.7)	42

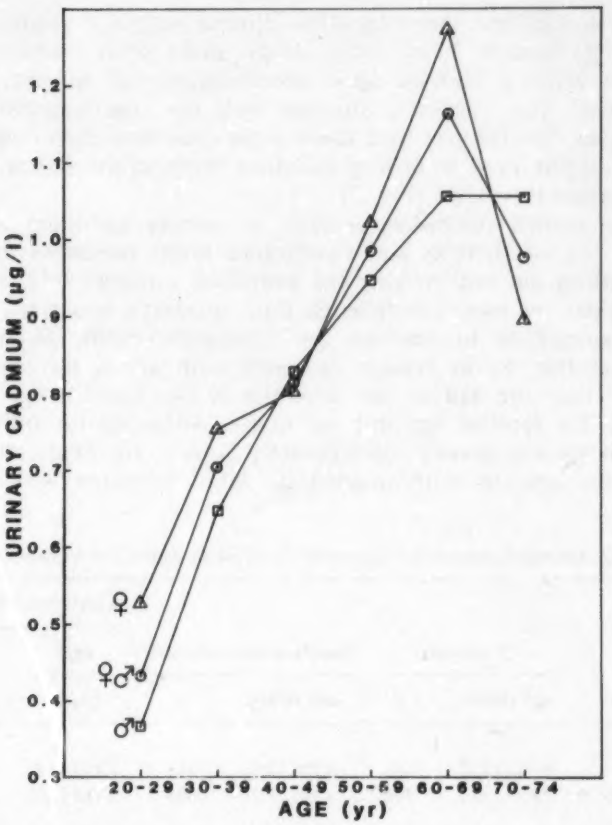

FIGURE 2. Geometric means of specific-gravity-adjusted urinary cadmium versus age.

TABLE 3. Polynomial Regression Coefficients of ln (Urinary Cadmium) Against Age

	Unadjusted		Specific-gravity-adjusted		Creatinine-adjusted	
Parameter	Coefficient	p	Coefficient	p	Coefficient	p
Male						
Intercept	−2.67	0.0001	−2.78	0.0001	−3.34	0.0001
Age	0.075	0.0001	0.092	0.0001	0.102	0.0001
(Age)^2	−0.00058	0.0052	0.00073	0.0001	−0.00081	0.0001
r^2	13.6%		21.0%		25.3%	
Female						
Intercept	−1.94	0.0001	−1.96	0.0001	−2.67	0.0001
Age	0.041	0.0482	0.066	0.0002	0.089	0.0001
(Age)^2	−0.00029	0.1939	−0.00053	0.0059	−0.00072	0.0001
r^2	5.1%		10.1%		16.9%	

further increases the explained variation and statistical significance, as compared with specific gravity adjusting, but this may be spurious, due to the fact that creatinine itself is a function of age (*vide infra*), and its variation is now incorporated into the dependent variable, i.e., urinary cadmium.

The nonparametric analysis (i.e., the substitute for ANOVA) showed both sex and age to be significant for all three forms of the urinary cadmium data. The results of multiple comparisons of cadmium levels among age groups are presented in Table 4. Multiple comparisons among age groups within each sex yielded similar patterns, i.e., an increase to the sixties and then a slight decrease. The slight increase in significant differences among age groups in the creatinine-adjusted data, compared with the specific-gravity-adjusted data, again may be a result of creatinine being a function of age.

Thus our data show urinary cadmium to be significantly higher in females than males, and to increase significantly with age, with the suggestion of a decrease in the eighth decade of life. The age relationship is consistent with previous studies (Kowal et al., 1979), and the trend is generally believed to reflect the increasing body burden of cadmium with age (Elinder et al., 1978; Lauwerys et al., 1976). Higher urinary cadmium in females than males has also been found by Elinder et al. (1978) in Sweden, and by Tsuchiya (1978) in Japan.

Urinary Beta$_2$-Microglobulin

The frequency distributions of levels of urinary beta$_2$-microglobulin, summed over both sexes and all ages, are tabulated in Table 5. As with cadmium, low levels predominate in the population, but a small fraction of the population may have relatively high levels. By checking the log-transformed variables (unadjusted, specific-gravity-adjusted, and creatinine-adjusted urinary beta$_2$-microglobulin) and their residuals from

TABLE 4. Multiple Comparisons of Urinary Cadmium Levels among Age Groups (Based on Kruskal-Wallis Rank Sums)

	Unadjusted		Specific-gravity-adjusted		Creatinine-adjusted	
Age	μg/l	Difference[a]	μg/l	Difference[a]	μg/g creatinine	Difference[a]
20–29	0.316	A	0.432	A	0.319	A
30–39	0.456	B	0.704	B	0.538	B
40–49	0.496	B	0.815	B	0.700	B C
50–59	0.608	B C	0.986	B C	0.896	C D
60–69	0.761	C	1.163	C	1.032	D
70–74	0.609	B C	0.977	B C	0.843	C D

[a]Means with the same letter are not significantly different, within adjustment type.

TABLE 5. Frequency (F) and Cumulative Frequency (CF) Distributions (%) of Urinary Beta$_2$-microglobulin

β_2-Microglobulin (μg/l or μg/g creatinine)	Unadjusted ($n = 977$)		Specific-gravity-adjusted ($n = 977$)		Creatinine-adjusted ($n = 976$)	
	F	CF	F	CF	F	CF
0-<10	8.8	8.8	4.8	4.8	6.0	6.0
10-<20	10.8	19.7	4.2	9.0	5.1	11.2
20-<30	8.2	27.8	3.9	12.9	4.6	15.8
30-<40	8.0	35.8	5.6	18.5	6.8	22.5
40-<50	6.6	42.4	4.1	22.6	6.3	28.8
50-<60	7.0	49.3	7.3	29.9	9.8	38.6
60-<70	6.7	56.0	6.8	36.6	9.4	48.1
70-<80	5.5	61.5	7.3	43.9	9.5	57.6
80-<90	5.2	66.7	6.7	50.6	7.4	65.0
90-<100	4.9	71.6	6.7	57.2	5.3	70.3
100-<110	3.3	74.9	5.7	62.9	4.1	74.4
110-<120	2.6	77.5	5.0	68.0	3.0	77.4
120-<130	2.5	79.9	4.0	72.0	3.1	80.4
130-<140	1.8	81.8	3.6	75.5	2.5	82.9
140-<150	2.5	84.2	3.0	78.5	2.0	84.9
150-<160	0.9	85.2	2.9	81.4	1.6	86.6
160-<170	1.5	86.7	1.8	83.2	1.4	88.0
170-<180	1.4	88.1	1.8	85.1	0.7	88.7
180-<190	0.7	88.8	1.1	86.2	1.2	90.0
190-<200	2.0	90.9	0.8	87.0	0.7	90.7
200-<220	1.0	91.9	2.3	89.3	0.8	91.5
220-<240	1.1	93.0	1.7	91.0	0.9	92.4
240-<260	0.7	93.8	0.6	91.6	0.5	92.9
260-<280	0.4	94.2	0.6	92.2	0.5	93.4
280-<300	0.6	94.8	1.0	93.2	0.6	94.1
300-<320	0.1	94.9	0.3	93.6	0.5	94.6
320-<340	0.6	95.5	0.7	94.3	0.5	95.1
340-<360	0.5	96.0	0.3	94.6	0.4	95.5
360-<380	0.1	96.1	0.4	95.0	0.1	95.6
380-<400	0.2	96.3	0.4	95.4	0.1	95.7
400-<500	1.1	97.4	1.0	96.4	1.1	96.8
500-<1000	1.3	98.8	2.1	98.6	1.7	98.6
1000-<2000	0.3	99.1	0.5	99.1	0.7	99.3
2000-<3000	0.4	99.5	0.3	99.4	0.1	99.4
3000-<4000	0.3	99.8	0.2	99.6	0.3	99.7
4000-<5000	0.2	100.0	0.1	99.7	0.1	99.8
5000-<6000	–	–	0	99.7	0	99.8
6000-<7000	–	–	0.1	99.8	0.1	99.9
7000-<8000	–	–	0.2	100.0	0	99.9
8000-<9000	–	–	–	–	0	99.9
9000-<10000	–	–	–	–	0	99.9
10000-<15000	–	–	–	–	0.1	100.0

ANOVA, it was shown that they are lognormally distributed (Fig. 3). Lognormal distributions have been found to be common in beta$_2$-microglobulin data (Kjellstrom and Piscator, 1977).

Ellis et al. (1981) have suggested the normal range of urinary beta$_2$-microglobulin to be up to 300 μg/l, and over 2000 μg/l to be suggestive of renal tubular damage. In our sample, using specific-gravity-adjusted data, 911 people (93.2%) fall in the normal range, 57 (5.8%) in the intermediate category, and 9 (0.9%) in the renal tubular damage range (all of these individuals but 2, in their sixties and seventies). Nogawa et al. (1979b) have suggested over 5000 μg/l to represent tubular proteinuria; 3 people (0.3%) fall in this range. The causes of these latter high levels in our sample are, of course, unknown at the present time. They are unlikely to be cadmium-caused, since all values of urinary beta$_2$-microglobulin over 300 μg/l are associated with urinary cadmium levels less than 6.72 μg/l, but could be the result of exposure to other heavy metals, certain antibiotics, Fanconi's syndrome, shock, fever, other nephrotoxic agents, or other renal disorders (Davey, 1979). People with leukemia or lymphoma often have elevated serum beta$_2$-microglobulin because of rapid cellular

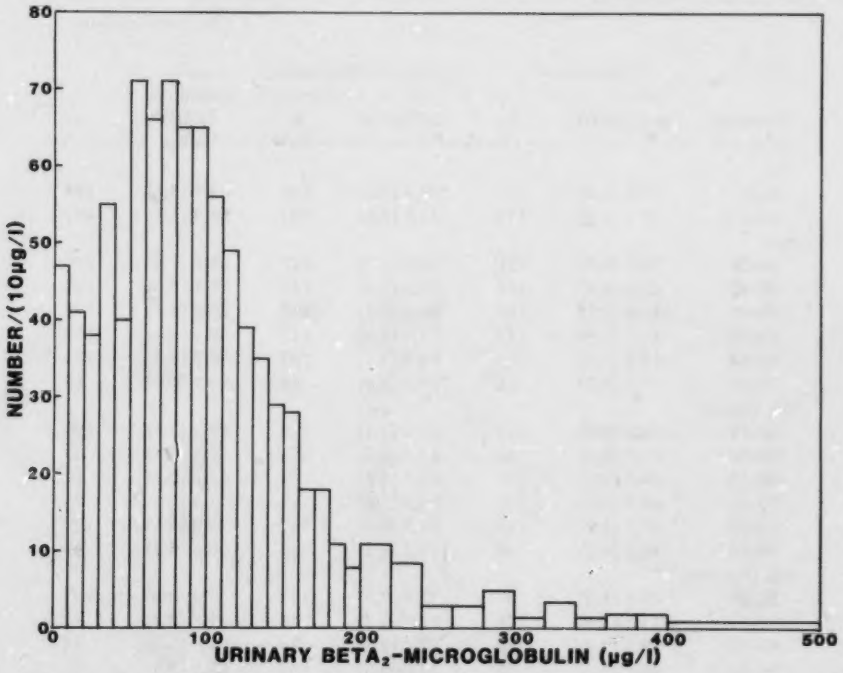

FIGURE 3. Frequency distribution of specific-gravity-adjusted urinary beta$_2$-microglobulin: $n = 977$, number in range 500–8000 μg/l = 35.

turnover and the protein's association with cell-membrane histocompatibility antigens (Mavligit et al., 1980), and this may elevate levels in the urine.

Using the upper limit of the 95% confidence interval, Pharmacia Diagnostics determined the normal upper limit of urinary $beta_2$-microglobulin to be 370 μg/l ($n = 107$) for ages 15–63. The same criterion for the normal upper limit of urinary $beta_2$-microglobulin results in our estimates of 413 μg/l ($n = 221$), 490 μg/l ($n = 141$), 472 μg/l ($n = 142$), 653 μg/l ($n = 117$), 1099 μg/l ($n = 268$), and 1451 μg/l ($n = 88$), for age groups 20–29, 30–39, 40–49, 50–59, 60–69, and 70–74, respectively.

The calculated geometric means and geometric standard deviations of urinary $beta_2$-microglobulin appear in Table 6, grouped by sex, age decade, and sex-age decade combinations. Our urinary $beta_2$-microglobulin (specific-gravity-adjusted) estimates of 89 (male) and 71 (female) μg/l are similar to those in Sweden, Japan, and Peru (Kjellstrom and Piscator, 1977). Stewart and Hughes (1981) have reported a level of 76 μg/l (95

TABLE 6. Geometric Means (and Geometric Standard Deviations) of Urinary $Beta_2$-microglobulin

	Unadjusted		Specific-gravity-adjusted		Creatinine-adjusted	
Parameter	μg/l (GSD)	*n*	μg/l (GSD)	*n*	μg/g creatinine (GSD)	*n*
Sex						
Male	67.8 (3.4)	484	89.4 (3.0)	484	68.1 (3.1)	484
Female	40.4 (3.5)	493	71.2 (3.0)	493	64.3 (3.1)	492
Age						
20–29	58.9 (2.8)	221	80.6 (2.3)	221	59.5 (2.4)	221
30–39	46.2 (3.4)	141	71.5 (2.7)	141	55.1 (2.6)	140
40–49	41.8 (3.2)	142	68.6 (2.7)	142	59.0 (2.5)	142
50–59	46.5 (3.4)	117	75.5 (3.0)	117	68.5 (3.1)	117
60–69	55.0 (4.1)	268	84.2 (3.7)	268	74.5 (3.8)	268
70–74	66.7 (4.0)	88	107.1 (3.8)	88	92.4 (4.0)	88
Age (males)						
20–29	70.6 (2.6)	120	87.2 (2.1)	120	62.2 (2.1)	120
30–39	67.0 (3.1)	63	87.5 (2.6)	63	59.5 (2.5)	63
40–49	60.8 (3.3)	69	82.4 (2.8)	69	64.2 (2.8)	69
50–59	60.7 (3.1)	55	83.8 (2.4)	55	67.9 (2.6)	55
60–69	68.7 (4.0)	131	91.9 (4.0)	131	75.0 (4.1)	131
70–74	80.3 (4.5)	46	111.2 (4.3)	46	86.4 (4.3)	46
Age (females)						
20–29	47.4 (3.0)	101	73.4 (2.5)	101	56.4 (2.6)	101
30–39	34.3 (3.3)	78	60.7 (2.7)	78	51.8 (2.7)	77
40–49	29.3 (2.8)	73	57.7 (2.4)	73	54.4 (2.2)	73
50–59	36.7 (3.6)	62	68.8 (3.5)	62	69.0 (3.5)	62
60–69	44.5 (4.0)	137	77.4 (3.5)	137	74.1 (3.5)	137
70–74	54.5 (3.5)	42	99.4 (3.8)	42	99.4 (3.8)	42

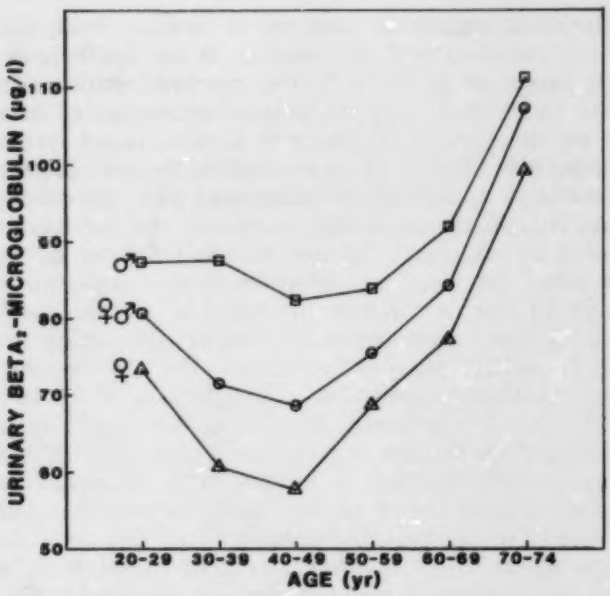

FIGURE 4. Geometric means of specific-gravity-adjusted urinary beta$_2$-microglobulin versus age.

μg/l at specific gravity 1.020) in British men, and Barltrop and Strehlow (1982) have reported a value, uncorrected for concentration, of 92 μg/l in an English village. Iwao et al. (1980) have reported a level of 118 μg/g creatinine in unexposed Japanese factory workers, almost double our level of urinary beta$_2$-microglobulin (64–68 μg/g creatinine).

As with the cadmium data, comparison among the urine concentration adjustment methods indicate that females have more dilute urine than males, and that females have lower urine creatinine than males. The specific-gravity-adjusted data suggest a U-shaped age effect, with a fall in urinary beta$_2$-microglobulin concentrations to a minimum in the forties, followed by a rise to the seventies (Fig. 4). The creatinine-normalized data show a similar curve, with the minimum occurring in the thirties. However, if one calculates the ratio of specific-gravity-adjusted to creatinine-adjusted age group means, one obtains the following series of ratios for the six age decades: 1.355, 1.298, 1.163, 1.102, 1.130, and 1.159. This suggests (since creatinine is in the numerator of this ratio) that creatinine also has a U-shaped age effect, with the minimum in the fifties. [The suggestion is confirmed below (see Table 9).] The high creatinine levels in the twenties and thirties would explain how the minimum for creatinine-adjusted beta$_2$-microglobulin is shifted to the left, i.e., from the forties to the thirties, thus obscuring the true age effect on urinary beta$_2$-microglobulin.

The polynomial regression analysis of urinary $beta_2$-microglobulin against urinary cadmium and age resulted in the coefficients and significance levels presented in Table 7. The decreased statistical significance and explained variation (r^2) in the concentration-adjusted data are probably caused by the removal of the urine dilution factor (present in each pair of unadjusted urinary $beta_2$-microglobulin and cadmium values) during concentration adjustment. In unadjusted data, the members of each pair of values contain the same dilution factor, and the resulting correlation is removed by adjustment for concentration. Overall, cadmium has an insignificant effect on $beta_2$-microglobulin in the concentration-adjusted data, although all four coefficients are positive. All age coefficients are negative, and all $(age)^2$ coefficients are positive, reinforcing the U-shaped effect of age on urinary $beta_2$-microglobulin. The specific-gravity-adjusted age and $(age)^2$ coefficients are statistically significant at the 10% level, but not at the 5% level. The creatinine-adjusted age and $(age)^2$ coefficients are not significant, perhaps because of creatinine's own relationship to age.

The nonparametric analysis of urinary $beta_2$-microglobulin (specific-gravity-adjusted) showed sex to be significant, but not age. However, age was almost significant in both the nonparametric test ($p = 0.0807$) and the parametric ANOVA ($p = 0.0561$). For creatinine-adjusted $beta_2$-microglobulin, sex was not significant, but age was significant. The results of multiple comparison of $beta_2$-microglobulin levels among age groups are presented in Table 8. The significance of the age effect in the creatinine-adjusted data is probably due to the high creatinine levels in the twenties and thirties lowering the $beta_2$-microglobulin values for those decades, so that they are much lower than the values for the sixties and seventies.

Our data show urinary $beta_2$-microglobulin (specific-gravity-adjusted)

TABLE 7. Polynomial Regression Coefficients of ln (Urinary $Beta_2$-microglobulin) against ln (Urinary Cadmium) and Age

	Unadjusted		Specific-gravity-adjusted		Creatinine-adjusted	
Parameter	Coefficient	p	Coefficient	p	Coefficient	p
Male						
Intercept	5.97	0.0001	5.24	0.0001	4.85	0.0001
ln Cd	0.332	0.0001	0.095	0.1014	0.130	0.0268
Age	−0.069	0.0027	−0.036	0.0995	−0.030	0.1729
$(Age)^2$	0.00066	0.0061	0.00039	0.0923	0.00035	0.1362
r^2	8.2%		1.1%		2.0%	
Female						
Intercept	5.43	0.0001	4.92	0.0001	4.22	0.0001
ln Cd	0.301	0.0001	0.048	0.3708	0.041	0.4568
Age	−0.072	0.0019	−0.037	0.0816	−0.014	0.5162
$(Age)^2$	0.00075	0.0024	0.00045	0.0501	0.00025	0.2797
r^2	8.3%		1.4%		2.5%	

TABLE 8. Multiple Comparisons of Urinary Beta$_2$-microglobulin Levels among Age Groups (Based on Kruskal-Wallis Rank Sums)

	Unadjusted		Specific-gravity-adjusted		Creatinine-adjusted	
Age	μg/l	Difference[a]	μg/l	Difference[a]	μg/g creatinine	Difference[a]
20–29	58.9	A	80.6	A	59.5	B
30–39	46.2	A B	71.5	A	55.1	B
40–49	41.8	B	68.6	A	59.0	A B
50–59	46.5	A B	75.5	A	68.5	A B
60–69	55.0	A B	84.2	A	74.5	A
70–74	66.7	A B	107.1	A	92.4	A

[a]Means with the same letter are not significantly different, within adjustment type.

to be significantly higher in males than females, and probably related to age. The level of statistical significance of the coefficients for negative age and positive $(age)^2$ in the regression models for both sexes (Table 7), and the values of the age-class means (Table 6), suggest a U-shaped age effect, with a minimum level in the fifth decade of life. The cause of such a phenomenon is conjectural—perhaps physical activity in youth and cadmium accumulation and/or kidney degeneration in age. The regression models indicate no significant increase in beta$_2$-microglobulin with increased cadmium. This is reasonable, since the urinary data consist predominantly of low—i.e., nonpathological—levels of both variables.

Kjellstrom and Piscator (1977) have found no effect of age on urinary beta$_2$-microglobulin in Sweden, in the range 5–65 years. Tsuchiya et al. (1979), on the other hand, have concluded that urinary beta$_2$-microglobulin increases with age in the range 50–70 years, an observation consistent with our results. They, however, found an increase in beta$_2$-microglobulin at low levels with increasing urinary cadmium.

Urinary Concentration Adjustment

In the previous two sections it was suggested that, while adjustment for urine concentration (or dilution or water content) is obviously necessary in any analysis of urine solutes, the use of creatinine as a basis may introduce extraneous variation which might obscure, or confound, the detection of the desired relationship. Specifically, there is evidence that (1) urinary creatinine is lower in females than males, and (2) urinary creatinine is a function of age, U-shaped, high in the twenties and thirties, with a minimum in the fifties, and rising again in the sixties and seventies. This suggests that any study (of adults) reporting creatinine-adjusted urine concentrations and involving both sexes and/or different ages is likely to have sex and/or age effects on creatinine confounded into the analysis.

That urinary creatinine and specific gravity are related is suggested by

TABLE 9. Multiple Comparisons of Urinary Specific Gravity Values and Creatinine Levels

Parameter	Specific gravity	Difference[a]	Unadjusted creatinine mg/l	Unadjusted creatinine Difference[a]	Specific-gravity-adjusted creatinine mg/l	Specific-gravity-adjusted creatinine Difference[a]
Sex						
Male	1.0170	A	1210	A	1394	A
Female	1.0134	B	829	B	1186	B
Age						
20–29	1.0166	A	1238	A	1424	A
30–39	1.0153	A B	1094	A B	1383	A B
40–49	1.0143	B	908	B	1244	B C
50–59	1.0146	B	877	B	1187	C
60–69	1.0151	B	947	B	1216	C
70–74	1.0142	B	917	B	1230	B C

[a]Means with the same letter are not significantly different, within comparison group.

their statistical correlation: a parametric Pearson correlation coefficient of 0.783 ($p = 0.0001$, $n = 981$), and a nonparametric Spearman correlation coefficient of 0.843 ($p = 0.0001$, $n = 981$). The use of creatinine for concentration adjustment, however, may introduce extraneous variation; this is confirmed by the results of the multiple comparisons presented in Table 9 (parametric for specific gravity, nonparametric for creatinine). All persons in their twenties have particularly concentrated urine, and females are confirmed to have significantly more dilute urine than males. These same trends are reflected in the unadjusted creatinine data, where both concentration effects and intrinsic sex-age effects on creatinine are confounded together. Removal of the concentration effects by specific-gravity-adjusting the urinary creatinine data reveals that the trends suggested above are indeed significant. Thus, we conclude that (1) urinary creatinine is lower in females than males and (2) urinary creatinine is a function of age, high in the twenties and thirties, decreasing to a minimum in the fifties and sixties, and then possibly rising slightly. As with $beta_2$-microglobulin, the cause of this age function is conjectural, again perhaps high muscle mass and physical activity in youth, and physiological changes in advancing age.

Bulusu et al. (1970) have found similar trends in urinary creatinine concentration—i.e., females lower than males, and creatinine excretion increasing with age to a maximum in the third decade, then decreasing to the eighth decade.

Numerous studies using multiple 24-h urine specimens from individuals, often under constant diet and standard conditions, have concluded that 24-h urinary creatinine excretion is not constant (Chattaway et al., 1969; Cryer and Sode, 1970; Edwards et al., 1969; Paterson, 1967; Rubin,

1971). Chattaway et al. (1969) found creatinine to increase with increasing meat content of diet, but added that other controlling factors were muscle mass, hormonal balance, mental state, physical activity, and tissue fluid and ion balance. The same pattern of temporal variation within individuals has been found in children, including increased urinary creatinine with increased nitrogen intake (Ritchey et al., 1973).

In an analysis of methods of data adjustment for urine concentration, Elkins et al. (1974) concluded that there was no general superiority of creatinine over specific gravity or osmolality. In an earlier publication (Elkins et al., 1966) they had concluded that osmolality had little practical advantage over specific gravity, except in cases of glucosuria or proteinuria, where specific gravity may be unduly elevated.

Ideally one would have data on the 24-h output of urine solutes. However, the collection of 24-h samples in occupational, epidemiological, or survey work is usually not practical, and, even when it is, questions on the accuracy of collection persist. The use of urinary creatinine to either estimate 24-h solute output or correct for urine concentration variations appears to be contraindicated by its variation with the sex and age of the individual, and by its temporal variation within the individual. Thus, specific gravity is the most straightforward and practical parameter to use in data adjustment for urine concentration.

REFERENCES

Barltrop, D., and Strehlow, C. D. 1982. Clinical and biochemical indices of cadmium exposure in the population of Shipham. *Proc. Third Int. Cadmium Conf.*, Miami, 1981, pp. 112–113. Cadmium Association, Cadmium Council, and International Lead Zinc Research Organization, London and New York.

Bernard, A., Buchet, J. P., Roels, H., Masson, P., and Lauwerys, R. 1979. Renal excretion of proteins and enzymes in workers exposed to cadmium. *Eur. J. Clin. Invest.* 9:11–22.

Bernard, A. M., Lauwerys, R. R., Starace, V., and Masson, P. L. 1980. Isolation of a new low molecular weight β_2-globulin from urine of a worker with chronic cadmium poisoning. *Biochem. Biophys. Res. Commun.* 93:535–543.

Bulusu, L., Hodgkinson, A., Nordin, B. E. C., and Peacock, M. 1970. Urinary excretion of calcium and creatinine in relation to age and body weight in normal subjects and patients with renal calculus. *Clin. Sci.* 38:601–612.

Chattaway, F. W., Hullin, R. P., and Odds, F. C. 1969. The variability of creatinine excretion in normal subjects, mental patients and pregnant women. *Clin. Chim. Acta* 26:567–576.

Cryer, P. E., and Sode, J. 1970. Variation in urinary creatinine excretion and its relationship to measurement of urinary 17-hydroxycorticosteroids. *Clin. Chem.* 16:1012–1015.

Davey, P. G. 1979. β_2-Microglobulin excretion and site of urinary-tract infection. *Lancet* 2:590–591.

Edwards, O. M., Bayliss, R. I. S., and Millen, S. 1969. Urinary creatinine excretion as an index of the completeness of 24-hour urine collections. *Lancet* 2:1165–1166.

Elinder, C.-G., Kjellstrom, T., Linnman, L., and Pershagen, G. 1978. Urinary excretion of cadmium and zinc among persons from Sweden. *Environ. Res.* 15:473–484.

Elkins, H. B., Pagnotto, L. D., and Richmond, M. 1966. The osmolality adjustment in urinalysis. *J. Occup. Med.* 8:528–531.

Elkins, H. B., Pagnotto, L. D., and Smith, H. L. 1974. Concentration adjustments in urinalysis. *Am. Ind. Hyg. Assoc. J.* 35:559–565

Ellis, K. J., Morgan, W. D., Zanzi, I., Yasumura, S., Vartsky, D., and Cohn, S. H. 1981. Critical concentrations of cadmium in human renal cortex: Dose-effect studies in cadmium smelter workers. *J. Toxicol. Environ. Health* 7:691–703.

Hollander, M., and Wolfe, D. A. 1973. *Nonparametric Statistical Methods.* New York: Wiley.

Iwao, S., Tsuchiya, K., and Sakurai, H. 1980. Serum and urinary beta-2-microglobulin among cadmium-exposed workers. *J. Occup. Med.* 22:399–402.

Kjellstrom, T., and Piscator, M. 1977. Quantitative analysis of β_2-microglobulin in urine as an indicator of renal tubular damage induced by cadmium. Pharmacia Diagnostics, Uppsala, Sweden.

Kowal, N. E., and Kraemer, D. F. 1982. Urinary cadmium and β_2-microglobulin levels of persons aged 20–74 years from a sub-sample of the national HANES II survey, 1976–1980. *Proc. Third Int. Cadmium Conf.*, Miami, 1981, pp. 119–122. Cadmium Association, Cadmium Council, and International Lead Zinc Research Organization, London and New York.

Kowal, N. E., Johnson, D. E., Kraemer, D. F., and Pahren, H. R. 1979. Normal levels of cadmium in diet, urine, blood, and tissues of inhabitants of the United States. *J. Toxicol. Environ. Health* 5:995–1014.

Lauwerys, R. R., Buchet, S. J. P., and Roels, H. 1976. The relationship between cadmium exposure on body burden and the concentration of cadmium in blood and urine in man. *Int. Arch. Occup. Environ. Health* 36:275–285.

Lauwerys, R., Roels, H., Regniers, M., Buchet, J. P., Bernard, A., and Goret, A. 1979. Significance of cadmium concentration in blood and in urine in workers exposed to cadmium. *Environ. Res.* 20:375–391.

Mavligit, G. M., Stuckey, S. E., Cabanillas, F. F., Keating, M. J., Tourtellotte, W. W., Schold, S. C., and Freireich, E. J. 1980. Diagnosis of leukemia or lymphoma in the central nervous system by beta$_2$-microglobulin determination. *N. Engl. J. Med.* 303:718–722.

Nogawa, K., Ishizaki, A., and Kobayashi, E. 1979a. A comparison between health effects of cadmium and cadmium concentration in urine among inhabitants of the itai-itai disease endemic district. *Environ. Res.* 18:397–409.

Nogawa, K., Kobayashi, E., and Honda, R. 1979b. A study of the relationship between cadmium concentrations in urine and renal effects of cadmium. *Environ. Health Perspect.* 28:161–168.

Paterson, N. 1967. Relative constancy of 24-hour urine volume and 24-hour creatinine output. *Clin. Chim. Acta* 18:57–58.

Poulik, M. D., Gold, P., and Shuster, J. 1979. β_2-Microglobulin: Methods and clinical application. *CRC Crit. Rev. Clin. Lab. Sci.* 10:225–245.

Ritchey, S. J., Derise, N. L., Abernathy, R. P., and Korslund, M. K. 1973. Variability of creatinine excretion in preadolescent girls consuming a wide range of dietary nitrogen. *Am. J. Clin. Nutr.* 26:690–695.

Roels, H. A., Lauwerys, R. R., Buchet, J.-P., Bernard, A., Chettle, D. R., Harvey, T. C., and Al-Haddad, I. K. 1981. *In vivo* measurement of liver and kidney cadmium in workers exposed to this metal: Its significance with respect to cadmium in blood and urine. *Environ. Res.* 26:217–240.

Rubin, R. T. 1971. Urine creatinine excretion: Variability and volume dependency during sleep deprivation. *Psychosom. Med.* 33:539–543.

Ryan, J. A., Pahren, H. R., and Lucas, J. B. 1982. Controlling cadmium in the human food chain: A review and rationale based on health effects. *Environ. Res.* 28:251–302.

Stewart, M., and Hughes, E. G. 1981. Urinary β_2-microglobulin in the biological monitoring of cadmium workers. *Br. J. Ind. Med.* 38:170–174.

Tsuchiya, K. 1978. Cadmium in human urine, feces, blood, hair, organs, and tissues. In *Cadmium Studies in Japan: A Review*, ed. K. Tsuchiya, pp. 37–43. New York: Elsevier/North Holland.

Tsuchiya, K., Iwao, S., Suguta, M., and Sakurai, H. 1979. Increased urinary β_2-microglobulin in cadmium exposure: Dose-effect relationship and biological significance of β_2-microglobulin. *Environ. Health Perspect.* 28:147–153.

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